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conclude  
(MPA)-Pro-Arg-Lys-Leu-Tyr-Asp-NH<sub>2</sub> (SEQ ID NO: 39);  
NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-( Nε-AEEA-MPA)-NH<sub>2</sub> (SEQ ID NO: 40); and  
NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-( Nε-AEEA<sub>n</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 41).

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**In the Sequence Listing**

Please enter the attached 22 pages of printed Sequence Listing as new pages 1-22 and remove the original attached Sequence Listing pages 1-9.

**REMARKS**

Reconsideration is respectfully requested. This amendment to the specification and the claims is made to identify sequences and Seq. ID. NOs that were inadvertently left out of the original sequence listing. Claims 19-21 have been amended. After entry of this amendment, claims 1-21 will be pending.

In response to the *Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures*, please find enclosed a paper and computer-readable copy of the Sequence Listing. To the best of the undersigned's knowledge, the printed Sequence Listing is identical to the Sequence Listing submitted in computer-readable form.

A copy of the *Notice to Comply* is attached hereto as required by United States Patent Office rules governing gene sequences.

Applicant notes that the computer-readable form of the Sequence Listing originally submitted did not comply with the requirements of 37 CFR 1.821-1.825. The format problems have been corrected with this response.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**". A deleted item is indicated by crossing out the item, e.g., ~~and~~, while an insertion is underlined.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 500862002200.

Respectfully submitted,

Dated: February 8, 2002

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Specification**

See attached replacement page Nos. 2, 3, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, and 61.

**In the Claims**

See attached claims 19-21.

**In the Claims:**

19. (Amended) A modified kringle 5 peptide selected from the group consisting of NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Lys-NH<sub>2</sub> (SEQ ID NO: 17); NAc-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-NH<sub>2</sub> (SEQ ID NO: 18); Nac-  
 5 Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-NH<sub>2</sub> (SEQ ID NO: 19); NAc-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Ala-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-NH<sub>2</sub> (SEQ ID NO: 20); NAc-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Lys-NH<sub>2</sub> (SEQ ID NO: 21); NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-(N<sub>ε</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 22);  
 10 (MPA-AEEA)-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 23) and (MPA)-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 24).

20.(Amended) A modified kringle 5 peptide selected from the group consisting of: NAc-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-(N<sub>ε</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 25); (MPA-AEEA)-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 26); (MPA)-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 27); NAc-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Ala-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-( N<sub>ε</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 28);  
 20 (MPA-AEEA)-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Ala-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 29); and (MPA)-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Ala-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 30).

25 21.(Amended) [20.] A modified kringle 5 peptide selected from the group consisting of NAc-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Lys-( N<sub>ε</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 31); (MPA-AEEA)-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-NH<sub>2</sub> (SEQ ID NO: 32); (MPA)-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-NH<sub>2</sub> (SEQ ID NO: 33);  
 30 NAc-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-( N<sub>ε</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 34); (MPA-AEEA)-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 35); (MPA)-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 36); NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Lys-( N<sub>ε</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 37);

(MPA-AEEA)-Pro-Arg-Lys-Leu-Tyr-Asp-NH<sub>2</sub> (SEQ ID NO: 38);

(MPA)-Pro-Arg-Lys-Leu-Tyr-Asp-NH<sub>2</sub> (SEQ ID NO: 39);

NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-( N<sub>ε</sub>-AEEA-MPA)-NH<sub>2</sub> (SEQ ID NO: 40); and

5 NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-( N<sub>ε</sub>-AEEA<sub>n</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 41).

endogeneous peptidases leading to very short plasma half-lives thereby reducing their usefulness as anti-angiogenic agents. As a result of their short half lives, peptides such as kringle 5 require constant infusion to reach adequate plasma levels sufficient for efficient therapy.

5 As a result, there is a need for long lasting anti-angiogenic peptides such as kringle 5. Such long lasting peptides would be useful in treating angiogenesis related diseases in mammals.

### **SUMMARY OF THE INVENTION**

10 In order to meet these needs, the present invention is directed to modified anti-angiogenic peptides. In particular, this invention is directed to modified kringle 5 peptides. The invention relates to novel chemically reactive derivatives of anti-angiogenic peptides that can react with available functionalities on mobile blood proteins to form covalent  
15 linkages. Specifically, the invention relates to novel chemically reactive derivatives of anti-angiogenic peptides such as kringle 5 peptides that can react with available functionalities on mobile blood proteins to form covalent linkages. The chemically reactive derivatives of the anti-angiogenic peptides are capable of forming a peptidase stabilized anti-  
20 angiogenic peptide.

The invention is directed to a derivative of an anti-angiogenic peptide such as a kringle 5 peptide where the derivative comprises a reactive group which reacts with amino groups, hydroxyl groups or thiol groups on blood proteins to form stable covalent bonds. In a preferred  
25 format, the anti-angiogenic peptides include succinimidyl or maleimido reactive groups.

The present invention relates to modified kringle 5 peptides and derivatives thereof and their use as anti-angiogenic agents. The kringle 5 peptides include reactive groups capable of forming a covalent bond  
30 with mobile blood proteins.

In particular, the present invention relates to the following modified kringle 5 peptides: NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Lys-NH<sub>2</sub> (SEQ ID NO: 17);

- NAc-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-NH<sub>2</sub> (SEQ ID NO: 18); Nac-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-NH<sub>2</sub> (SEQ ID NO: 19); NAc-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Ala-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-NH<sub>2</sub> (SEQ ID NO: 20); NAc-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Lys-NH<sub>2</sub> (SEQ ID NO: 21);
- 5 NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-(N<sub>ε</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 22); (MPA-AEEA)-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 23); (MPA)-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 24); NAc-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-( N<sub>ε</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 25);
- 10 (MPA-AEEA)-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 26); (MPA)-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 27); NAc-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Ala-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-( N<sub>ε</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 28);
- 15 (MPA-AEEA)-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Ala-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 29); (MPA)-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Ala-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 30); NAc-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Lys-( N<sub>ε</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 31);
- 20 (MPA-AEEA)-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-NH<sub>2</sub> (SEQ ID NO: 32); (MPA)-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-NH<sub>2</sub> (SEQ ID NO: 33); NAc-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-( N<sub>ε</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 34); (MPA-AEEA)-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 35);
- 25 (MPA)-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub> (SEQ ID NO: 36); NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Lys-( N<sub>ε</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 37); (MPA-AEEA)-Pro-Arg-Lys-Leu-Tyr-Asp-NH<sub>2</sub> (SEQ ID NO: 38); (MPA)-Pro-Arg-Lys-Leu-Tyr-Asp-NH<sub>2</sub> (SEQ ID NO: 39); NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-( N<sub>ε</sub>-AEEA-MPA)-NH<sub>2</sub> (SEQ ID NO: 40);
- 30 NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-( N<sub>ε</sub>-AEEA<sub>n</sub>-MPA)-NH<sub>2</sub> (SEQ ID NO: 41); and other modified kringle 5 peptides.

The modified anti-angiogenic peptides find use in the treatment of angiogenesis in humans.

**Preparation of NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Lys-NH<sub>2</sub>.3TFA (SEQ ID NO: 42)**

Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin:

5 Fmoc-Lys(Boc)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH.

Deblocking of the Fmoc group the the N-terminal of the resin-bound amino acid was performed with 20% piperidine in DMF for about 15-20 minutes. Coupling of the acetic acid was performed under conditions

10 similar to amino acid coupling. Final cleavage from the resin was performed using cleavage mixture as described above. The product was isolated by precipitation and purified by preparative HPLC to afford the desired product as a white solid upon lyophilization

**Example 2**

**Preparation of NAc-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-NH<sub>2</sub>.3TFA (SEQ ID NO: 43)**

Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin:

20 Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH.

Deblocking of the Fmoc group the the N-terminal of the resin-bound amino acid was performed with 20% piperidine in DMF for about 15-20 minutes. Coupling of the acetic acid was performed under conditions

25 similar to amino acid coupling. Final cleavage from the resin was performed using cleavage mixture as described above. The product was isolated by precipitation and purified by preparative HPLC to afford the desired product as a white solid upon lyophilization.

**Example 3**

**Preparation of Nac-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-L u-Tyr-Asp-Tyr-Lys-NH<sub>2</sub>.3TFA (SEQ ID NO: 44)**

Using automated peptide synthesis, the following protected



amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Tyr(tBu)OH. Deblocking of the Fmoc group the the N-terminal of the resin-bound amino acid was performed with 20% piperidine in DMF for about 15-20 minutes. Coupling of the acetic acid was performed under conditions similar to amino acid coupling. Final cleavage from the resin was performed using cleavage mixture as described above. The product was isolated by precipitation and purified by preparative HPLC to afford the desired product as a white solid upon lyophilization.

#### Example 4

#### 15 Preparation of NAc-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Ala-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-NH<sub>2</sub>.4TFA (SEQ ID NO: 45)

Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Ala-OH, Fmoc-Trp-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Pbf)-OH. Deblocking of the Fmoc group the the N-terminal of the resin-bound amino acid was performed with 20% piperidine in DMF for about 15-20 minutes. Coupling of the acetic acid was performed under conditions similar to amino acid coupling. Final cleavage from the resin was performed using cleavage mixture as described above. The product was isolated by precipitation and purified by preparative HPLC to afford the desired product as a white solid upon lyophilization.

**Example 5****Preparation of NAc-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Lys-NH<sub>2</sub>.2TFA (SEQ ID NO: 46)**

5           Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Trp-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Pbf)-OH.  
10        Deblocking of the Fmoc group the the N-terminal of the resin-bound amino acid was performed with 20% piperidine in DMF for about 15-20 minutes. Coupling of the acetic acid was performed under conditions similar to amino acid coupling. Final cleavage from the resin was  
15        performed using cleavage mixture as described above. The product was isolated by precipitation and purified by preparative HPLC to afford the desired product as a white solid upon lyophilization

**Example 6****Preparation of NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-(N $\epsilon$ -MPA)-NH<sub>2</sub>.2TFA (SEQ ID NO: 47)**

20           Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Aloc)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH,  
25        Fmoc-Pro-OH. Deblocking of the Fmoc group the the N-terminal of the resin-bound amino acid was performed with 20% piperidine in DMF for about 15-20 minutes. Coupling of the acetic acid was performed under conditions similar to amino acid coupling. Final cleavage from the resin was performed using cleavage mixture as described above. The  
30        product was isolated by precipitation and purified by preparative HPLC to afford the desired product as a white solid upon lyophilization.

          The selective deprotection of the Lys(Aloc) group was performed

manually and accomplished by treating the resin with a solution of 3 eq of  $\text{Pd}(\text{PPh}_3)_4$  dissolved in 5 mL of  $\text{CHCl}_3$ :NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin was then washed with  $\text{CHCl}_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis was then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Resin cleavage and product isolation was performed using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold  $\text{Et}_2\text{O}$  (Step 4). The product was purified by preparative reversed phase HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in  $\text{H}_2\text{O}$  (A) and 0.045% TFA in  $\text{CH}_3\text{CN}$  (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at 214 and 254 nm.

#### Example 7

##### **Preparation of (MPA-AEEA)-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr- $\text{NH}_2$ .2TFA (SEQ ID NO: 48)**

Solid phase peptide synthesis of the modified Kringle 5 peptide on a 100  $\mu$ mole scale was performed on a Symphony Peptide Synthesizer using Fmoc protected Rink Amide MBHA resin, Fmoc protected amino acids, O-benzotriazol-1-yl-*N*, *N*', *N*'-tetramethyluronium hexafluorophosphate (HBTU) in *N,N*-dimethylformamide (DMF) solution and activation with *N*-methyl morpholine (NMM), and piperidine deprotection of Fmoc groups (Step 1).

Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Tyr(*t*Bu)OH, Fmoc-Asp(O*t*Bu)-OH, Fmoc-Tyr(*t*Bu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH. The deprotection of the terminal Fmoc group is accomplished using 20% piperidine (Step 2) followed by the coupling of Fmoc-AEEA. Deprotection of the resulting Fmoc-AEEA-peptide with piperidine 20% in DMF allow for the subsequent addition of the 3-MPA

(Step 3). Resin cleavage and product isolation was performed using 86% TFA/5% TIS/5% H<sub>2</sub>O/2% thioanisole and 2% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system using a Dynamax C<sub>18</sub>, 60Å, 8 µm, 21 mm x 25 cm column equipped with a Dynamax C<sub>18</sub>, 60Å, 8 µm guard module, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm. The product had >95% purity as determined by RP-HPLC mass spectrometry using a Hewlett Packard LCMS-1100 series spectrometer equipped with a diode array detector and using electro-spray ionization.

#### Example 8

##### Preparation of (MPA)-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub>·2TFA (SEQ ID NO: 49)

Solid phase peptide synthesis of the modified Kringle 5 peptide on a 100 µmole scale was performed on a Symphony Peptide Synthesizer using Fmoc protected Rink Amide MBHA resin, Fmoc protected amino acids, O-benzotriazol-1-yl-*N, N, N, N*-tetramethyluronium hexafluorophosphate (HBTU) in *N,N*-dimethylformamide (DMF) solution and activation with *N*-methyl morpholine (NMM), and piperidine deprotection of Fmoc groups (Step 1). Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH. The deprotection of the terminal Fmoc group is accomplished using 20% piperidine (Step 2) followed by the coupling of 3-MPA (Step 3). Resin cleavage and product isolation was performed using 86% TFA/5% TIS/5% H<sub>2</sub>O/2% thioanisole and 2% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system using a Dynamax C<sub>18</sub>, 60Å, 8 µm, 21 mm x 25 cm column equipped with a

Dynamax C<sub>18</sub>, 60Å, 8 µm guard module, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm. The product had >95% purity as determined by RP-HPLC mass spectrometry using a Hewlett Packard LCMS-1100 series spectrometer equipped with a diode array detector and using electro-spray ionization.

### Example 9

#### Preparation of NAc-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-( N<sub>ε</sub>-MPA)-NH<sub>2</sub>.2TFA (SEQ ID NO: 50)

Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Aloc)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Tyr(tBu)OH. Deblocking of the Fmoc group the the N-terminal of the resin-bound amino acid was performed with 20% piperidine in DMF for about 15-20 minutes. Final cleavage from the resin was performed using cleavage mixture as described above. The product was isolated by precipitation and purified by preparative HPLC to afford the desired product as a white solid upon lyophilization

The selective deprotection of the Lys(Aloc) group was performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin was then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis was then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Resin cleavage and product isolation was performed using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5

mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at 214 and 254 nm.

### Example 10

#### 5      **Preparation of (MPA-AEEA)-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub>.2TFA (SEQ ID NO: 51)**

     Solid phase peptide synthesis of the modified Kringle 5 peptide on a 100  $\mu$ mole scale was performed on a Symphony Peptide Synthesizer using Fmoc protected Rink Amide MBHA resin, Fmoc  
10      protected amino acids, O-benzotriazol-1-yl-*N, N, N', N'*-tetramethyluronium hexafluorophosphate (HBTU) in *N,N*-dimethylformamide (DMF) solution and activation with *N*-methyl morpholine (NMM), and piperidine deprotection of Fmoc groups (Step 1).

     Using automated peptide synthesis, the following protected  
15      amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Tyr(tBu)OH. The deprotection of the terminal Fmoc group is  
20      accomplished using 20% piperidine (Step 2) followed by the coupling of Fmoc-AEEA. Deprotection of the resulting Fmoc-AEEA-peptide with piperidine 20% in DMF allow for the subsequent addition of the 3-MPA (Step 3). Resin cleavage and product isolation was performed using  
25      86% TFA/5% TIS/5% H<sub>2</sub>O/2% thioanisole and 2% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system using a Dynamax C<sub>18</sub>, 60Å, 8  $\mu$ m, 21 mm x 25 cm column equipped with a Dynamax C<sub>18</sub>, 60Å, 8  $\mu$ m guard module, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and  
30      254 nm. The product had >95% purity as determined by RP-HPLC mass spectrometry using a Hewlett Packard LCMS-1100 series spectrometer equipped with a diode array detector and using electro-

spray ionization.

### Example 11

#### Preparation of (MPA)-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub>.2TFA (SEQ ID NO: 52)

5 Solid phase peptide synthesis of the modified Kringle 5 peptide on a 100  $\mu$ mole scale was performed on a Symphony Peptide Synthesizer using Fmoc protected Rink Amide MBHA resin, Fmoc protected amino acids, O-benzotriazol-1-yl-*N, N, N, N*-tetramethyl-uronium hexafluorophosphate (HBTU) in *N,N*-dimethylformamide (DMF) solution and activation with *N*-methyl morpholine (NMM), and piperidine deprotection of Fmoc groups (Step 1 Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)OH, 10 Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Tyr(tBu)OH. The deprotection of the terminal Fmoc group is accomplished using 20% piperidine (Step 2) followed by the coupling of 3-MPA (Step 3). Resin cleavage and product isolation was performed using 86% TFA/5% 20 TIS/5% H<sub>2</sub>O/2% thioanisole and 2% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system using a Dynamax C<sub>18</sub>, 60Å, 8  $\mu$ m, 21 mm x 25 cm column 25 equipped with a Dynamax C<sub>18</sub>, 60Å, 8  $\mu$ m guard module, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254 nm. The product had >95% purity as determined by RP-HPLC mass spectrometry using a Hewlett Packard LCMS-1100 series spectrometer equipped with a diode array detector and using electro-spray ionization.

**Example 12****Preparation of NAc-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Ala-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-(N<sub>ε</sub>-MPA)-NH<sub>2</sub>.3TFA (SEQ ID NO: 53)**

5           Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Aloc)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Ala-OH, Fmoc-Trp-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Pbf)-OH. Deblocking of the Fmoc group the the N-terminal of the resin-bound amino acid was performed with 20% piperidine in DMF for about 15-20 minutes. Coupling of the acetic acid was performed under conditions similar to amino acid coupling. Final cleavage from the resin was performed using cleavage mixture as described above. The product was isolated by precipitation and purified by preparative HPLC to afford the desired product as a white solid upon lyophilization.

20           The selective deprotection of the Lys(Aloc) group was performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin was then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis was then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Resin cleavage and product isolation was performed using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5



mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at 8 214 and 254 nm.

### Example 13

#### 5 **Preparation of (MPA-AEEA)-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Ala-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub>.3TFA (SEQ ID NO: 54)**

Solid phase peptide synthesis of the modified Kringle 5 peptide on a 100  $\mu$ mole scale was performed on a Symphony Peptide  
 10 Synthesizer using Fmoc protected Rink Amide MBHA resin, Fmoc protected amino acids, O-benzotriazol-1-yl-*N, N, N, N*-tetramethyluronium hexafluorophosphate (HBTU) in *N,N*-dimethylformamide (DMF) solution and activation with *N*-methyl morpholine (NMM), and piperidine deprotection of Fmoc groups (Step 1).

15 Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Tyr(*t*Bu)OH, Fmoc-Asp(*Ot*Bu)-OH, Fmoc-Tyr(*t*Bu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(*t*Bu)-OH, Fmoc-Thr(*t*Bu)-  
 20 OH, Fmoc-Tyr(*t*Bu)OH, Fmoc-Ala-OH, Fmoc-Trp-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Asp(*Ot*Bu)-OH, Fmoc-Gly-OH, Fmoc-Asp(*Ot*Bu)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Pbf)-OH. The deprotection of the terminal Fmoc group is accomplished using 20% piperidine (Step 2) followed by the coupling of  
 25 Fmoc-AEEA. Deprotection of the resulting Fmoc-AEEA-peptide with piperidine 20% in DMF allow for the subsequent addition of the 3-MPA (Step 3). Resin cleavage and product isolation was performed using 86% TFA/5% TIS/5% H<sub>2</sub>O/2% thioanisole and 2% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by  
 30 preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system using a Dynamax C<sub>18</sub>, 60Å, 8  $\mu$ m, 21 mm x 25 cm column equipped with a Dynamax C<sub>18</sub>, 60Å, 8  $\mu$ m guard module, 21 mm

x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254 nm. The product had >95% purity as determined by RP-HPLC mass spectrometry using a Hewlett Packard LCMS-1100 series spectrometer equipped with a diode array detector and using electro-spray ionization.

#### Example 14

##### **Preparation of (MPA)-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Ala-Tyr-Thr-Thr-Asn-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub>.3TFA (SEQ ID NO: 55)**

Solid phase peptide synthesis of the modified Kringle 5 peptide on a 100  $\mu$ mole scale was performed on a Symphony Peptide Synthesizer using Fmoc protected Rink Amide MBHA resin, Fmoc protected amino acids, O-benzotriazol-1-yl-*N, N, N', N'*-tetramethyluronium hexafluorophosphate (HBTU) in *N,N*-dimethylformamide (DMF) solution and activation with *N*-methyl morpholine (NMM), and piperidine deprotection of Fmoc groups (Step 1). Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Ala-OH, Fmoc-Trp-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Pbf)-OH.

The deprotection of the terminal Fmoc group is accomplished using 20% piperidine (Step 2) followed by the coupling of 3-MPA (Step 3). Resin cleavage and product isolation was performed using 86% TFA/5% TIS/5% H<sub>2</sub>O/2% thioanisole and 2% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system using a Dynamax C<sub>18</sub>, 60Å, 8  $\mu$ m, 21 mm x 25 cm column equipped with a Dynamax C<sub>18</sub>, 60Å, 8  $\mu$ m guard module, 21 mm

x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254 nm. The product had >95% purity as determined by RP-HPLC mass spectrometry using a Hewlett Packard LCMS-1100 series spectrometer equipped with a diode array detector and using electro-spray ionization.

5

### Example 15

#### Preparation of NAc-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-Lys-(N $\epsilon$ -MPA)-NH $_2$ .TFA (SEQ ID NO: 56)

10 Solid phase peptide synthesis of the modified Kringle 5 peptide on a 100  $\mu$ mole scale was performed on a Symphony Peptide Synthesizer using Fmoc protected Rink Amide MBHA resin, Fmoc protected amino acids, O-benzotriazol-1-yl-*N,N,N,N*-tetramethyluronium hexafluorophosphate (HBTU) in *N,N*-dimethylformamide (DMF) solution and activation with *N*-methyl morpholine (NMM), and piperidine deprotection of Fmoc groups (Step 1).

15 Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Aloc)-OH, Fmoc-Trp-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Pbf)-OH. 20 The selective deprotection of the Lys(Aloc) group was performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh $_3$ ) $_4$  dissolved in 5 mL of CHCl $_3$ :NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin was then washed with CHCl $_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). 25 The synthesis was then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Resin cleavage and product isolation was performed using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et $_2$ O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) 30 preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H $_2$ O (A) and 0.045% TFA in CH $_3$ CN (B)) over 180 min at 9.5

mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at 214 and 254 nm.

### Example 16

#### 5      **Preparation of (MPA-AEEA)-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-NH<sub>2</sub>.TFA (SEQ ID NO: 57)**

10      Solid phase peptide synthesis of the modified Kringle 5 peptide on a 100  $\mu$ mole scale was performed on a Symphony Peptide Synthesizer using Fmoc protected Rink Amide MBHA resin, Fmoc protected amino acids, O-benzotriazol-1-yl-*N, N, N', N'*-tetramethyluronium hexafluorophosphate (HBTU) in *N,N*-dimethylformamide (DMF) solution and activation with *N*-methyl morpholine (NMM), and piperidine deprotection of Fmoc groups (Step 1).

15      Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Trp-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Arg(Pbf)-OH. The deprotection of the terminal Fmoc group is accomplished using  
20      20% piperidine (Step 2) followed by the coupling of Fmoc-AEEA. Deprotection of the resulting Fmoc-AEEA-peptide with piperidine 20% in DMF allow for the subsequent addition of the 3-MPA (Step 3). Resin cleavage and product isolation was performed using 86% TFA/5% TIS/5% H<sub>2</sub>O/2% thioanisole and 2% phenol, followed by precipitation by  
25      dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system using a Dynamax C<sub>18</sub>, 60Å, 8  $\mu$ m, 21 mm x 25 cm column equipped with a Dynamax C<sub>18</sub>, 60Å, 8  $\mu$ m guard module, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254  
30      nm. The product had >95% purity as determined by RP-HPLC mass spectrometry using a Hewlett Packard LCMS-1100 series spectrometer equipped with a diode array detector and using electro-spray ionization.

**Example 17****Preparation of (MPA)-Arg-Asn-Pro-Asp-Gly-Asp-Val-Gly-Gly-Pro-Trp-NH<sub>2</sub>.TFA (SEQ ID NO: 58)**

5 Solid phase peptide synthesis of the modified Kringle 5 peptide  
on a 100  $\mu$ mole scale was performed on a Symphony Peptide  
Synthesizer using Fmoc protected Rink Amide MBHA resin, Fmoc  
protected amino acids, O-benzotriazol-1-yl-*N, N, N, N*-tetramethyl-  
uronium hexafluorophosphate (HBTU) in *N,N*-dimethylformamide (DMF)  
solution and activation with *N*-methyl morpholine (NMM), and piperidine  
10 deprotection of Fmoc groups (Step 1). Using automated peptide  
synthesis, the following protected amino acids were sequentially added  
to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Trp-OH, Fmoc-  
Pro-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-  
OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Pro-OH, Fmoc-  
15 Asn(Trt)-OH, Fmoc-Arg(Pbf)-OH. The deprotection of the terminal  
Fmoc group is accomplished using 20% piperidine (Step 2) followed by  
the coupling of 3-MPA (Step 3). Resin cleavage and product isolation  
was performed using 86% TFA/5% TIS/5% H<sub>2</sub>O/2% thioanisole and 2%  
phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The  
20 product was purified by preparative reverse phase HPLC using a Varian  
(Rainin) preparative binary HPLC system using a Dynamax C<sub>18</sub>, 60Å, 8  
 $\mu$ m, 21 mm x 25 cm column equipped with a Dynamax C<sub>18</sub>, 60Å, 8  $\mu$ m  
guard module, 21 mm x 25 cm column and UV detector (Varian  
Dynamax UVD II) at  $\lambda$  214 and 254 nm. The product had >95% purity  
25 as determined by RP-HPLC mass spectrometry using a Hewlett Packard  
LCMS-1100 series spectrometer equipped with a diode array detector  
and using electro-spray ionization.

**Example 18****Preparation of NAc-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-(N<sub>ε</sub>-MPA)-NH<sub>2</sub>.2TFA (SEQ ID NO: 59)**

Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Aloc)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH. Deblocking of the Fmoc group the the N-terminal of the resin-bound amino acid was performed with 20% piperidine in DMF for about 15-20 minutes. Coupling of the acetic acid was performed under conditions similar to amino acid coupling. Final cleavage from the resin was performed using cleavage mixture as described above. The product was isolated by precipitation and purified by preparative HPLC to afford the desired product as a white solid upon lyophilization.

The selective deprotection of the Lys(Aloc) group was performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin was then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis was then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Resin cleavage and product isolation was performed using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at 8 214 and 254 nm.

**Example 19****Pr eparation of (MPA-AEEA)-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub>.2TFA (SEQ ID NO: 60)**

Solid phase peptide synthesis of the modified Kringle 5 peptide

on a 100  $\mu$ mole scale was performed on a Symphony Peptide Synthesizer using Fmoc protected Rink Amide MBHA resin, Fmoc protected amino acids, O-benzotriazol-1-yl-*N, N, N', N'*-tetramethyluronium hexafluorophosphate (HBTU) in *N,N*-dimethylformamide (DMF) solution and activation with *N*-methyl morpholine (NMM), and piperidine deprotection of Fmoc groups (Step 1).

Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Tyr(*t*Bu)OH, Fmoc-Asp(*Ot*Bu)-OH, Fmoc-Tyr(*t*Bu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH. The deprotection of the terminal Fmoc group is accomplished using 20% piperidine (Step 2) followed by the coupling of Fmoc-AEEA. Deprotection of the resulting Fmoc-AEEA-peptide with piperidine 20% in DMF allow for the subsequent addition of the 3-MPA (Step 3). Resin cleavage and product isolation was performed using 86% TFA/5% TIS/5% H<sub>2</sub>O/2% thioanisole and 2% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system using a Dynamax C<sub>18</sub>, 60Å, 8  $\mu$ m, 21 mm x 25 cm column equipped with a Dynamax C<sub>18</sub>, 60Å, 8  $\mu$ m guard module, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254 nm. The product had >95% purity as determined by RP-HPLC mass spectrometry using a Hewlett Packard LCMS-1100 series spectrometer equipped with a diode array detector and using electro-spray ionization

### Example 20

#### Preparation of (MPA)-Arg-Lys-Leu-Tyr-Asp-Tyr-NH<sub>2</sub>.2TFA (SEQ ID NO: 61)

Solid phase peptide synthesis of the modified Kringle 5 peptide on a 100  $\mu$ mole scale was performed on a Symphony Peptide Synthesizer using Fmoc protected Rink Amide MBHA resin, Fmoc protected amino acids, O-benzotriazol-1-yl-*N, N, N', N'*-tetramethyluronium hexafluorophosphate (HBTU) in *N,N*-dimethylformamide (DMF)

solution and activation with *N*-methyl morpholine (NMM), and piperidine deprotection of Fmoc groups (Step 1). Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH. The deprotection of the terminal Fmoc group is accomplished using 20% piperidine (Step 2) followed by the coupling of 3-MPA (Step 3). Resin cleavage and product isolation was performed using 86% TFA/5% TIS/5% H<sub>2</sub>O/2% thioanisole and 2% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system using a Dynamax C<sub>18</sub>, 60Å, 8 µm, 21 mm x 25 cm column equipped with a Dynamax C<sub>18</sub>, 60Å, 8 µm guard module, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm. The product had >95% purity as determined by RP-HPLC mass spectrometry using a Hewlett Packard LCMS-1100 series spectrometer equipped with a diode array detector and using electro-spray ionization.

## Example 21

### **Preparation of NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Lys-(N<sub>ε</sub>-MPA)-NH<sub>2</sub>.2TFA (SEQ ID NO: 62)**

Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Aloc)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH. Deblocking of the Fmoc group the the N-terminal of the resin-bound amino acid was performed with 20% piperidine in DMF for about 15-20 minutes. Coupling of the acetic acid was performed under conditions similar to amino acid coupling. Final cleavage from the resin was performed using cleavage mixture as described above. The product was isolated by precipitation and purified by preparative HPLC to afford



the desired product as a white solid upon lyophilization.

The selective deprotection of the Lys(Aloc) group was performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin was then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis was then re-automated for the addition of the 3-maleimidopropionic acid (Step 3). Resin cleavage and product isolation was performed using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10 μ phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at 214 and 254 nm.

## Example 22

### Preparation of (MPA-AEEA)-Pro-Arg-Lys-Leu-Tyr-Asp-NH<sub>2</sub>.2TFA

#### (SEQ ID NO: 63)

Solid phase peptide synthesis of the modified Kringle 5 peptide on a 100 μmole scale was performed on a Symphony Peptide Synthesizer using Fmoc protected Rink Amide MBHA resin, Fmoc protected amino acids, O-benzotriazol-1-yl-*N, N, N', N'*-tetramethyluronium hexafluorophosphate (HBTU) in *N,N*-dimethylformamide (DMF) solution and activation with *N*-methyl morpholine (NMM), and piperidine deprotection of Fmoc groups (Step 1).

Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH (Step 1). The deprotection of the terminal Fmoc group is accomplished using 20% piperidine (Step 2) followed by the coupling of Fmoc-AEEA. Deprotection of the resulting Fmoc-AEEA-peptide with piperidine 20% in

DMF allow for the subsequent addition of the 3-MPA (Step 3). Resin cleavage and product isolation was performed using 86% TFA/5% TIS/5% H<sub>2</sub>O/2% thioanisole and 2% phenol, followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system using a Dynamax C<sub>18</sub>, 60Å, 8 µm, 21 mm x 25 cm column equipped with a Dynamax C<sub>18</sub>, 60Å, 8 µm guard module, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at λ 214 and 254 nm. The product had >95% purity as determined by RP-HPLC mass spectrometry using a Hewlett Packard LCMS-1100 series spectrometer equipped with a diode array detector and using electrospray ionization.

### Example 23

#### 15 Preparation of (MPA)-Pro-Arg-Lys-Leu-Tyr-Asp-NH<sub>2</sub>·2TFA (SEQ ID NO: 64)

Solid phase peptide synthesis of the modified Kringle 5 peptide on a 100 µmole scale was performed on a Symphony Peptide Synthesizer using Fmoc protected Rink Amide MBHA resin, Fmoc protected amino acids, O-benzotriazol-1-yl-*N, N, N, N*-tetramethyluronium hexafluorophosphate (HBTU) in *N,N*-dimethylformamide (DMF) solution and activation with *N*-methyl morpholine (NMM), and piperidine deprotection of Fmoc groups (Step 1). Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Asp(OtBu)-OH, 20 Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH. The deprotection of the terminal Fmoc group is accomplished using 20% piperidine (Step 2) followed by the coupling of 3-MPA (Step 3). Resin cleavage and product isolation was performed using 86% TFA/5% TIS/5% H<sub>2</sub>O/2% thioanisole and 2% phenol, 25 followed by precipitation by dry-ice cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system using a Dynamax C<sub>18</sub>, 60Å, 8 µm, 21 mm x 25 cm column equipped with a Dynamax C<sub>18</sub>, 60Å, 8 µm guard 30

module, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254 nm. The product had >95% purity as determined by RP-HPLC mass spectrometry using a Hewlett Packard LCMS-1100 series spectrometer equipped with a diode array detector and using electro-spray ionization.

#### Example 24

##### **Preparation of NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-(N $\epsilon$ -AEEA-MPA)-NH $_2$ .2TFA (SEQ ID NO: 65)**

Using automated peptide synthesis, the following protected amino acids were sequentially added to Rink Amide MBHA resin: Fmoc-Lys(Aloc)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Asp(OtBu)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Pro-OH (Step 1). Deblocking of the Fmoc group at the N-terminal of the resin-bound amino acid was performed with 20% piperidine in DMF for about 15-20 minutes. Coupling of the acetic acid was performed under conditions similar to amino acid coupling. The selective deprotection of the Lys(Aloc) group was performed manually and accomplished by treating the resin with a solution of 3 eq of Pd(PPh $_3$ ) $_4$  dissolved in 5 mL of CHCl $_3$ :NMM:HOAc (18:1:0.5) for 2 h (Step 2). The resin was then washed with CHCl $_3$  (6 x 5 mL), 20% HOAc in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The synthesis was then re-automated for the addition of the AEEA (aminoethoxyethoxyacetic acid) group and of the 3-maleimidopropionic acid (MPA) (Step 3). Resin cleavage and product isolation was performed using 85% TFA/5% TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice cold Et $_2$ O (Step 4). The product was purified by preparative reverse phase HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 30-55% B (0.045% TFA in H $_2$ O (A) and 0.045% TFA in CH $_3$ CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna 10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian Dynamax UVD II) at  $\lambda$  214 and 254 nm.

**Example 25****Preparation of NAc-Pro-Arg-Lys-Leu-Tyr-Asp-Tyr-Lys-(N $\epsilon$ -AEEA<sub>n</sub>-MPA)-NH<sub>2</sub>.2TFA (SEQ ID NO: 66)**

Using automated peptide synthesis, the following protected  
5 amino acids were sequentially added to Rink Amide MBHA resin:  
Fmoc-Lys(Aloc)-OH, Fmoc-Tyr(tBu)OH, Fmoc-Asp(OtBu)-OH, Fmoc-  
Tyr(tBu)OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH,  
Fmoc-Pro-OH (Step 1). Deblocking of the Fmoc group at the N-  
terminal of the resin-bound amino acid was performed with 20%  
10 piperidine in DMF for about 15-20 minutes. Coupling of the acetic acid  
was performed under conditions similar to amino acid coupling.

The selective deprotection of the Lys(Aloc) group was performed  
manually and accomplished by treating the resin with a solution of 3 eq  
of Pd(PPh<sub>3</sub>)<sub>4</sub> dissolved in 5 mL of CHCl<sub>3</sub>:NMM:HOAc (18:1:0.5) for 2 h  
15 (Step 2). The resin was then washed with CHCl<sub>3</sub> (6 x 5 mL), 20% HOAc  
in DCM (6 x 5 mL), DCM (6 x 5 mL), and DMF (6 x 5 mL). The  
synthesis was then re-automated for the addition The synthesis was  
then re-automated for the addition of n AEEA (aminoethoxyethoxyacetic  
acid) groups and of the 3-maleimidopropionic acid (MPA) (Step 3).  
20 Resin cleavage and product isolation was performed using 85% TFA/5%  
TIS/5% thioanisole and 5% phenol, followed by precipitation by dry-ice  
cold Et<sub>2</sub>O (Step 4). The product was purified by preparative reverse  
phase HPLC using a Varian (Rainin) preparative binary HPLC system:  
gradient elution of 30-55% B (0.045% TFA in H<sub>2</sub>O (A) and 0.045% TFA  
25 in CH<sub>3</sub>CN (B)) over 180 min at 9.5 mL/min using a Phenomenex Luna  
10  $\mu$  phenyl-hexyl, 21 mm x 25 cm column and UV detector (Varian  
Dynamax UVD II) at 214 and 254 nm.

## Example 26

### Peptide Stability Assay

5 A peptide stability assay was performed. (MPA)-Pro-Arg-Lys-Leu-Tyr-Asp-Lys-NH<sub>2</sub>. 2TFA (SEQ ID NO: 67) was synthesized as described above and was identified MPA-K5. The non-modified counterpart peptide Pro-Arg-Lys-Leu-Tyr-Asp-Lys-NH<sub>2</sub> (SEQ ID NO: 12) was also synthesized as described above without the addition of 3-MPA and identified as K5.

10 K5 (MW = 1260.18, 918.12 freebase) was prepared as a 100 mM stock solution in water. MPA-K5 (MW = 1411.17, 1069.11 freebase) was prepared as a 100 mM stock solution in water. Human Serum Albumin (HSA) was obtained as a 25% solution (ca 250 mg/ml, 3.75 mM) as Albutein® available from Alpha Therapeutic. Human plasma was obtained from Golden West Biologicals.

15

#### a. Stability of K5 in human plasma

K5 was prepared as a 1µM solution and dissolved in 25% human serum albumin. The mixture was then incubated at 37°C in the presence of human plasma to final concentration of 160 mM K5. 20 Aliquots of 100 µl were withdrawn from the plasma at 0, 4 hours and 24 hours. The 100 µl aliquots were mixed with 100 µl of blocking solution (5 vol. 5%ZnSO<sub>4</sub>/3 vol. Acetonitrile/2 vol. Methanol) in order to precipitate all proteins. The sample was centrifuged for 5 min at 10,000 g and the supernatant containing the peptide was recovered and filtered 25 through a 0.22 µm filter. The presence of free intact K5 peptide was assayed by the HPLC/MS. The HPLC parameters for detection of K5 peptide in serum were as follows.

30 The HPLC method was as follows: A Vydac C18 250 X 4.6 mm, 5 µ particle size column was utilized. The column temperature was 30°C with a flow rate of 0.5 ml/min. Mobile Phase A was 0.1% TFA/water. Mobile Phase B was 0.1% TFA/acetonitrile. The injection volume was 10µl.

The gradient was as follows: